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(71) Applicant *(for all designated States except US)*: GROPEP PTY. LTD. [AU/AU]; 39 Winwood Street, Thebarton, S.A. 5031 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : BALLARD, Francis, John [AU/AU]; 23 Lowan Avenue, Glenalta, S.A. 5031 (AU). FRANCIS, Geoffrey, Leonard [AU/AU]; 9 Joyleen Court, Athelstone, S.A. 5076 (AU). REGESTER, Geoffrey, Owen [AU/AU]; Unit 5, 2 Cassie Street, Collinswood, S.A. 5081 (AU).

(74) Agent: PHILLIPS, ORMONDE & FITZPATRICK; 367 Collins Street, Melbourne VIC 3000 (AU).

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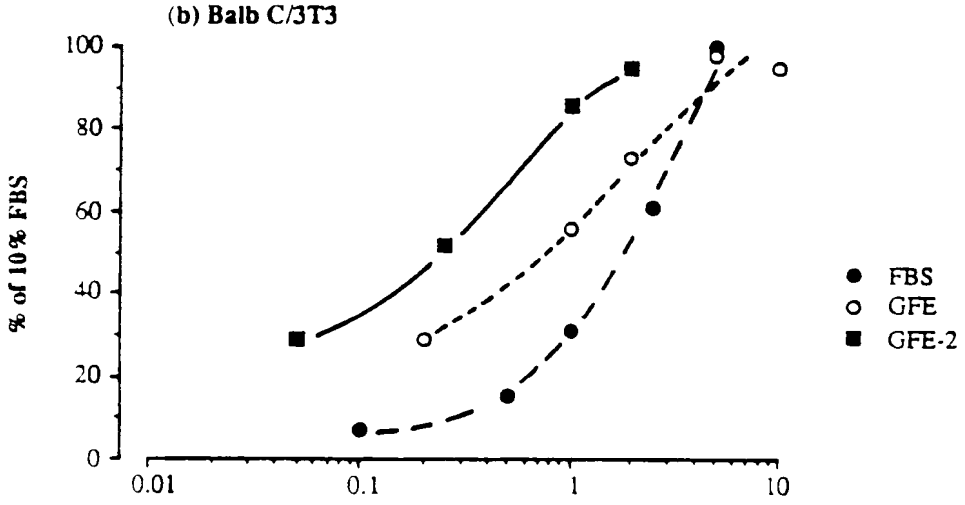

(57) Abstract

A milk product extract composiiton including a plurality of cell growth stimulating factors, extracted from milk product, in concentrated form; said factors having basic to approximately neutral isoelectric points. Cell culture compositions and pharmaceutical or veterinary compositions including the above milk product extract. Methods for preparing and using the same.

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GROWTH-PROMOTING AGENT

This invention relates to the growth of animal cells in a cell culture composition. More specifically it relates to the provision of a cell culture composition including a cheese whey extract composition.

Animal cells are grown in culture to provide a number of Pharmaceutical, Diagnostic and Veterinary products including Human vaccines, Lymphokines, Hormones, Monoclonal antibodies, Other Pharmaceutically active protein products, Veterinary hormones and for Research and Development and Diagnostic purposes.

The growth of animal cells requires a defined isotonic medium that contains salts, nutrients, lipid precursors, nucleic acid precursors, vitamins and amino acids that are formulated to mimic the medium that would normally bathe those cells in vivo. Examples in common use include Eagle's Minimal Essential Medium, Dulbecco's-modified Eagle's Minimal Essential Medium (DMEM), Medium 199, RPMI 1640 medium and Ham's F12 Medium. However, virtually no animal cells will grow in such a medium, but require the co-addition of serum. Fetal bovine serum is frequently used as it is more effective than serum obtained from post-natal animals and it contains only minimal concentrations of immunoglobulins which otherwise could have undesirable effects.

The supply of fetal bovine serum is limited by the number of pregnant cows slaughtered. It also has undesirable lot-to-lot variations and may include toxins. Particular concern surrounds its use for the eventual production of recombinant proteins and other pharmaceuticals for human use because the serum may also contain viruses that are harmful to humans and may be carried through a purification protocol that yields the desirable product. Principally for these reasons, extensive efforts have been directed towards the replacement of serum by pure ingredients. Examples of such ingredients are growth factors, hormones and cell attachment factors. Unfortunately, the requirements of each cell type being grown are different and are difficult

to establish. Frequently it has not proved possible to achieve equivalent growth properties or equivalent yields of cell products with "serum-free" media as can be obtained with medium containing fetal bovine serum.

The limited availability of fetal bovine serum, its lot-to-lot variability, its resultant considerable cost as well as the deficiencies of "serum-free" media described above have prompted the investigation of other biological fluids as potential replacements in cell culture media. Some progress has been reported in the prior art with bovine milk and bovine colostrum as evidenced by the following selected reports: M. Klagsbrun: "Human milk stimulates DNA synthesis and cell proliferation in cultured fibroblasts" (Proc. Natl. Acad. Sci. USA 75, 5057, 1978); M. Klagsbrun & J. Neumann: "The serum-free growth of Balb/c 3T3 cells in medium supplemented with bovine colostrum" (J. Supramol. Struct. 11, 349, 1979).

The prior art also includes U.S. Patent 4,440,860 to M. Klagsbrun which describes "compositions and methods for promoting cell growth featuring, in one aspect, cell culture media containing milk or colostrum and fibronectin; fibronectin is preferably pre-coated onto the culture substrate" and Japan Patent JP 59166879 to Morinaga "A culture medium for cell incubation - containing milk or milk components". Ultrafiltrates of milk whey have also been used to support the growth of cultured cells, as in European Patent 86401911.2 to G. Linden et al. "Fractions de lait, Procedee d'obtention de ces fractions et milleux de culturo cellulaires renfermant ces fractions" and O. Damerdji et al. "Utilization of whey fractions as a substitute for fetal calf serum in culture media" (Biotech. Tech. 2, 235, 1988).

Despite this progress a successful alternative to fetal bovine serum is yet to be located.

It is accordingly an object of the present invention to overcome, or at least alleviate one or more of the difficulties or deficiencies related to the prior art.

Accordingly in a first aspect of the present invention there is provided a milk product extract

composition including a plurality of cell growth stimulating factors, extracted from milk product, in concentrated form; said factors having basic to approximately neutral isoelectric points.

By the term "milk product" we mean an extract from human or animal milk product in which the salt and/or main protein constituents thereof are reduced or eliminated. Examples of milk extract include cheese whey extracts, skim milk extract and acid (casein) whey.

The present invention will be more fully described with reference to the preferred cheese whey extracts. However, this is illustrative only and should not be taken as a restriction on the generality of the invention.

Preferably the milk product extract composition is a cheese whey extract composition.

The cheese whey extract composition may be formed from cheese whey wherein the salt and/or main protein constituents thereof are reduced or eliminated.

The milk product extract composition may include less than approximately 1% w/w salt, based on the total weight of the composition. The milk product extract may include less than approximately 0.5% w/w casein, alpha lactalbumin, beta lactoglobulin, immunoglobulin or albumin, based on the total weight of the composition.

The milk product extract composition according to this aspect of the present invention may be utilised in the promotion of cell growth and proliferation <u>in vitro</u> as discussed below. The milk product extract composition may be utilised in stimulation of surface wound repair <u>in vivo</u>, in mammals as discussed below.

Surprisingly, the milk product extract composition may support the growth of animal cells at lower protein concentrations than achieved with fetal bovine serum, yet with an efficacy comparable to fetal bovine serum for several cell types.

Alternatively, the cheese whey extract may be used as a supplement to media containing low concentrations of fetal bovine serum in order to achieve

better growth rates of cultured cells and to conserve the use of fetal bovine serum.

Cheese whey is a by-product of the cheese industry that has had essentially all the fat and casein removed during cheese manufacture. At the present state of the art cheese whey is essentially valueless, and indeed it may represent a net cost to the industry since it is a potential pollutant.

Cheese whey for example is a low protein, high salt product available in tonne amounts from cheese manufacture. The main protein constituents present in cheese whey are alpha lactalbumin (αLA) and beta lactoglobulin (ßLG), which usually account for more than 90% of the proteins present. Significant amounts of serum albumin, immunoglobulins and residual casein may be present. All of these proteins have acidic isoelectric points. In contrast, the main protein factors that stimulate the growth of animal cells have basic isoelectric points. Examples include the growth factors basic FGF, IGF-I, des(1-3)IGF-I and PDGF. It is postulated that the extraction of the basic factors present in milk products such as cheese whey in the virtual absence of the otherwise abundant acidic proteins may account for the surprising efficacy of the milk product extract composition.

Accordingly in a further aspect of the present invention, there is provided a method for preparing a milk product extract composition including a plurality of cell growth stimulating factors, extracted from milk product in concentrated form; said factors having basic to approximately neutral isoelectric points, which method includes

providing

a source of milk product;

a cationic exchange resin; and

a buffer solution;

contacting the milk product with the cation exchange resin such that the more basic components of the milk product are absorbed thereon;

eluting the cationic exchange resin with the buffer solution; and

filtering the eluate to remove salt therefrom.

The desorption of the basic proteins from the ion exchange resin leads to a preparation enriched in cell growth stimulating factors. The eluate may be concentrated and filtered utilising any suitable technique. The eluate may be concentrated for example by conventional ultrafiltration methods or other procedures to yield a mixture of proteins which supports the growth of animal cells when added to protein-free media such as DMEM.

The source of milk product may be a milk product filtrate substantially free of insoluble material. Accordingly the preparation method may include the preliminary step of

filtering the milk product to remove insoluble materials therefrom.

The milk product may be filtered through a suitable sieve. The milk product may be filtered through a hollow fiber cartridge of defined porosity.

The cationic exchange resin may be of any suitable type. A Sepharose - based cation exchange gel may be used. The contacting step may be conducted at neutral to basic pH. The contacting step may be conducted at a pH of approximately 6.5 to 8.0.

The cationic exchange resin may be equilibrated with a suitable buffer at a pH of approximately 6.5 to 8.0. An aqueous sodium citrate buffer may be used. The elution steps may be conducted utilising a suitable eluate. A salt solution may be used. A buffered saline solution may be used.

Thus in a preferred form of this aspect of the present invention the method of preparing a milk product extract composition may include treating milk product sequentially by:

subjecting the milk product to a filtration step, to remove insoluble materials therefrom;

adjusting the pH of the filtrate to between

approximately 6.5 and 8.0;

contacting the filtrate with a cationic exchange resin;

eluting from the cation exchange resin at high ionic strength and high pH with a suitable buffer solution; and

subjecting the eluate to a concentration step and diafiltration step to remove salt therefrom.

Alternatively, the elution from the cation exchange resin is achieved at high ionic strength but without adjusting pH, such that the cell growth stimulating factors are recovered.

In this embodiment the cell growth stimulating factors are eluted with less extraneous protein.

In a further aspect of the isolation of a suitable extract from cheese whey, the eluant may be treated at high temperature and centrifuged. This modification removes additional protein. Accordingly, the method may further include subjecting the eluant to a heat treatment to reduce the content of extraneous protein.

The milk product extract composition may be sterilized and optionally freeze-dried for storage. The freeze-dried material may be dissolved in sterile saline for addition to cells in culture.

In a further aspect of the present invention there is provided a cell culture composition including an effective amount of a milk product extract composition including

a plurality of cell growth stimulating factors, extracted from milk product, in concentrated form; said factors having basic to approximately neutral isoelectric points; and

a culture medium.

The culture medium may be a substantially protein-free isotonic culture medium. The substantially protein-free isotonic culture medium may be Dulbecco's-modified Eagle's minimal Essential Medium (DMEM).

It has been found that an approximately

equivalent growth rate of human skin fibroblasts to that achieved with 5% Fetal Bovine Serum may be achieved with approximately 20 µg of cell growth stimulating factors extracted from cheese whey according to the preferred aspect of the present invention per 100 µl of medium.

Alternatively a small but effective amount of fetal bovine serum may be utilised as the culture medium. It has been found that the addition of approximately 25 µg of cell growth stimulating factors per 100 µl of medium containing approximately 2% fetal bovine serum will increase the growth rate of Balb C/3T3 cells to that rate otherwise achieved with 10% fetal bovine serum.

Other additions may be made to the medium, depending on the cell type, including growth factors, attachment factors or low amounts of serum.

In a preferred form, the present invention provides a cell culture composition, as described above, wherein the milk product extract is present in media at a protein concentration of approximately 10 to 20,000 micrograms per ml, preferably 100 to 2,000 micrograms per ml.

Accordingly in a still further aspect of the present invention there is provided a method for culturing cells which method includes

providing

a source of animal cells; and

a cell culture composition including an effective amount of a milk product extract composition including

a plurality of cell growth stimulating factors, extracted from milk product, in concentrated form; said factors having basic to approximately neutral isoelectric points; and

a substantially protein-free isotonic culture medium; and

culturing the cells in the cell culture composition for a time sufficient, and at a temperature sufficient to achieve a predetermined cell concentration.

The cell culture method may be conducted at ambient temperature or above. A temperature in the range of approximately 35 to 40°C may be used. The cell culture process may be conducted in an incubator, for example a humidified incubator.

The cell culture method may be conducted on any suitable surface or in suspension. Tissue culture plates may be used.

The cell culture method may continue for a period of approximately 1 to 5 days depending on the cell concentration desired.

Although the method in particular applies to the growth of animal cells in vitro, it can also be applied to animals, including humans, that have surface wounds.

Accordingly, in a further aspect, the present invention provides a pharmaceutical or veterinary composition for the treatment of surface wounds, which composition includes:

an effective amount of a milk product extract composition including a plurality of cell growth promoting factors, extracted from milk product in concentrated form; said factors having basic to approximately neutral isoelectric points; and

a pharmaceutically or veterinarily-acceptable diluent, carrier or excipient therefor.

The pharmaceutical or veterinary composition may further include an effective amount of at least one active ingredient.

The at least one active ingredient may be selected from antibiotics, antiseptics, other growth promotants, anaesthetics, and the like, and mixtures thereof.

The pharmaceutical or veterinary composition may be adapted for administration in any suitable manner. The composition may be adapted for internal or topical administration. The composition may be in an oral, injectable or topical form. Topical administration is preferred. The composition may take the form of a wash, lotion, cream, ointment or gel.

There are no limitations to the type of surface wound that may be treated, and these include, but are not limited to burns, ulcers, lacerations and penetrations.

Accordingly, in a further aspect of the present invention there is provided a method of treating surface wounds in animals, including humans, which method includes administering to the patient to be treated an effective amount of a pharmaceutical or veterinary composition, which composition includes

an effective amount of a milk product extract composition including a plurality of cell growth promoting factors, extracted from milk product in concentrated form; said factors having basic to approximately neutral isoelectric points; and

a pharmaceutically or veterinarily-acceptable diluent, carrier or excipient therefor.

The method can also be applied to animals, including humans, that have gastrointestinal injuries, diseases or ulcers.

Accordingly, in a further aspect, the present invention provides a pharmaceutical or veterinary composition for the treatment of gastrointestinal injuries, diseases or ulcers, which composition includes:

an effective amount of a milk product extract composition including a plurality of cell growth promoting factors, extracted from milk product in concentrated form; said factors having basic to approximately neutral isoelectric points; and

a pharmaceutically or veterinarily-acceptable diluent, carrier or excipient therefor.

There are no limitations to the type of gastrointestinal injury, disease or ulcer that may be treated.

Accordingly, in a still further aspect of the present invention, there is provided a method for the treatment of gastrointestinal injuries, diseases or ulcers, which method includes administering to the patient to be treated an effective amount of a pharmaceutical or veterinary composition, which composition includes

an effective amount of a milk product extract composition including cell growth promoting factors, extracted from milk product in concentrated form and having a basic to approximately neutral isoelectric point; and

a pharmaceutically or veterinarily acceptable diluent, carrier or excipient therefor.

The present invention will now be more fully described with repsect to the following examples. It should be understood, however, that the description following is illustrative only, and should not be taken in any way as a restriction on the generality of the invention described above.

EXAMPLE 1

Preparation of a fraction from cheese whey (GFE) that is enriched in growth-promoting activity

Pasteurized whey obtained as an end product of cheese manufacture was filtered through a 10 micron screen and a 0.2 micron Sartorius Microsart Sartocon II module to remove solids. The ultrafiltrate was adjusted to pH 6.5 and applied to a column of S-Sepharose Fast Flow S cation exchange resin (Pharmacia) that had been equilibrated with 50 mM sodium citrate buffer at pH 6.5. After washing the column with the same buffer the absorbed material was eluted by a solution of 1M NaCl containing 0.25 M NH_4OH. This eluate was diafiltered against water until the conductivity reached 0 µs and then concentrated by ultrafiltration; both processes using a 3KDa-excluding membrane. The resultant preparation was freeze-dried to produce the "GFE" product.

A preparation from 30 litres of cheese whey containing 18g protein yielded a GFE extract containing 2.66 g protein.

EXAMPLE 2

Preparation of a fraction from cheese whey that is enriched in growth-promoting activity and depleted in extraneous protein including lactoferrin (GFE-2)

Pasteurized whey was filtered and applied to a column of S-Sepharose and the column washed as in Example

1. Elution was accomplished with a solution containing 0.4M NaCl added to 10mM sodium citrate pH6.5. This GFE-2 was diafiltered against water, concentrated and freeze-dried as described in Example 1.

A preparation from 30 litres of cheese whey which contained 18g protein yielded a GFE-2 extract containing 0.56g protein.

EXAMPLE 3

Preparation of a modified GFE-2 fraction that is also depleted in extraneous protein including lactoperoxidase (GFE-3)

The freeze-dried GFE-2 (Example 2) was dissolved at a concentration of 25 mg/ml and heated at 80°C for 2.5 min. The heated sample was cooled rapidly and centrifuged. The clear supernatant was passed through a 0.22 µm filter before use. This solution contained 50% of the protein present in GFE-2 and approximately 10% lactoperoxidase.

EXAMPLE 4

Stimulation of the growth of cultured cells by cheese whey extracts (Examples 1, 2) compared with fetal bovine serum

Prior to addition to culture media, the freeze-dried powders (GFE, GFE-2) were first suspended in Dulbecco's Phosphate-buffered saline and sterilised by passage through a 0.22 µm filter.

This example utilises the cell lines L6 (rat myoblast), Balb C/3T3 (mouse fibroblast) and SF1972 (human diploid skin fibroblast).

Each cell line was subcultured on to 96-place tissue culture plates in Dulbecco-Modified Eagles's Minimal Essential Medium (DMEM) containing 5% fetal bovine serum and left in a 5% CO_2, 37°C, humidified incubator overnight to ensure attachment of the cells. Sterile techniques were used throughout. The plates were thoroughly washed in DMEM to remove any residual serum and the whey extract (GFE or GFE-2) or fetal bovine serum (FBS) added at the indicated concentrations. The total volume in each well was 0.1 ml at 37°C, 5% CO_2 and 100% humidity.

After a further 2 days the plates were washed, fixed and the cell numbers quantified using an automated methylene blue method (M.H. Oliver et al., J. Cell Sci. 92, 513, 1989). Growth is expressed as the percentage increase in absorbance units relative to the increse in absorbance produced by growing the cells in DMEM containing 5% fetal bovine serum (Figure 1).

This example shows that in all three cell lines GFE and GFE-2 stimulate growth as well as fetal bovine serum. Moreover, in Balb C/3T3 and SF1972 cells GFE-2 is active at approximately one tenth the protein content as fetal bovine serum.

EXAMPLE 5

Stimulation of the growth of cultured cells by extracts of cheese whey depleted in extraneous protein including lactoperoxidase (GFE-3, Example 3) compared with GFE-2 (Example 2)

The experimental details were exactly as described in Example 4 except that the data are expressed as the protein content (µg/100µl well) that achieved the same growth response as was achieved with 5% fetal bovine serum (see Table 1).

TABLE 1

Growth of Cells in the presence of GFE-2 or GFE-3

Cell Type	Extract	Concentration (µg/100µl) achieving growth equivalent to 5% fetal bovine serum
L6	GFE-2	100
	GFE-3	63
Balb C/3T3	GFE-2	15
	GFE-3	6
SF1972	GFE-2	8
	GFE-3	4

Clearly less GFE-3 is required to stimulate growth than GFE-2. Also since 5% fetal bovine serum has a

protein content of 250 µg/100µl, both GFE-2 and GFE-3 are very substantially more potent than 5% fetal bovine serum, especially for Balb C/3T3 cells and human skin fibroblasts (SF1972).

EXAMPLE 6

Growth effects of cultured cells produced by supplementing medium containing 2% fetal bovine serum with GFE-2 extracts (Example 2)

The experimental details were exactly as described in Example 4 except that the human lung fibroblast line (HEL) replaced the human skin fibroblast line (SF1972). Data are expressed as absorbances achieved after growth of the cells for 2 days (see Table 2).

TABLE 2

Growth of Cells with GFE-2 added in the presence of 2% fetal bovine serum

Fetal Bovine Serum (%)	GFE-2 (µg/100µl)	Increases in absorbance		
		L6 cells	Balb C/3T3 cells	HEL cells
2	0	0.618	0.126	0.165
5	0	0.998	0.270	0.210
10	0	1.309	0.502	0.345
2	5	1.010	0.294	0.322
2	25	1.108	0.585	0.388
2	50	1.157	0.698	0.389
2	100	1.370	0.799	0.374

This experiment demonstrates that low amounts of GFE-2 added to medium containing only 2% fetal bovine serum can increase the growth rate to that achieved with 10% fetal bovine serum. The approximate amount of GFE-2 required to achieve this growth enhancement was 100µg/100µl in L6 cells, 25µg/100µl in Balb C/3T3 cells and only 5µg/100µl in HEL cells. Such an

enhancement represents a very substantial saving of fetal bovine serum.

Finally, it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

Claims :

1. A milk product extract composition including a plurality of cell growth stimulating factors, extracted from milk product, in concentrated form; said factors having basic to approximately neutral isoelectric points.

2. A composition according to claim 1, wherein the milk product extract composition includes less than approximately 0.5% w/w casein, alpha lactalbumin, beta lactoglobulin, immunoglobulin or albumin, based on the total weight of the composition.

3. A composition according to claim 2, wherein the residual extraneous protein content of the milk product is further reduced.

4. A composition according to claim 3 wherein the milk product extract composition is a cheese whey extract composition.

5. A method for preparing a milk product extract composition including a plurality of cell growth stimulating factors, extracted from milk product, in concentrated form; said factors having basic to approximately neutral isoelectric points, which method includes

providing

a source of milk product;

a cationic exchange resin; and

a buffer solution;

contacting the milk product with the cation exchange resin such that the more basic components of the milk product are absorbed thereon;

eluting the cationic exchange resin with the buffer solution; and

filtering the eluate to remove salt therefrom.

6. A method according to claim 5, further including treating milk product sequentially by

subjecting the milk product to a filtration step, to remove insoluble materials therefrom;

adjusting the pH of the filtrate to between approximately 6.5 and 8.0;

contacting the filtrate with a cationic exchange resin;

eluting from the cation exchange resin at high ionic strength and high pH with a suitable buffer solution; and

subjecting the eluate to a concentration step and diafiltration step to remove salt therefrom.

7. A method according to claim 6, the elution step is conducted without adjusting pH, such that cell growth stimulating factors are recovered.

8. A method according to claim 7, further including subjecting the eluant to a heat treatment to reduce extraneous proteins.

9. A cell composition including an effective amount of a milk product extract composition including

a plurality of cell growth stimulating factors, extracted from milk product, in concentrated form; said factors having basic to approximately neutral isoelectric points; and

a culture medium.

10. A cell composition according to claim 9 wherein the culture medium is a substantially protein-free culture medium.

11. A cell composition according to claim 9 wherein the culture medium contains fetal bovine serum.

12. A cell culture composition according to claim 10, wherein the extraneous protein content of the milk product is further reduced.

13. A cell culture composition according to claim 12, wherein the milk product extract composition is present at a protein concentration of approximately 10 to 20,000 micrograms per ml of culture medium.

14. A method for culturing cells which method includes providing

a source of animal cells; and

a cell culture composition including an effective amount of a milk product extract composition including

a plurality of cell growth stimulating factors, extracted from milk product, in concentrated form; said factors having basic

to approximately neutral isoelectric points; and

a substantially protein-free isotonic culture medium; and

culturing the cells in the cell culture composition for a time sufficient, and at a temperature sufficient to achieve a predetermined cell concentration.

15. A method according to claim 14, wherein the cells are cultured at a temperature in the range of approximately 35°C to 40°C for a period of approximately 1 to 5 days.

16. A pharmaceutical or veterinary composition for the treatment of surface wounds, which composition includes:

an effective amount of a milk product extract composition including a plurality of cell growth promoting factors, extracted from milk product in concentrated form; said factors having basic to approximately neutral isoelectric points; and

a pharmaceutically or veterinarily-acceptable diluent, carrier or excipient therefor.

17. A composition according to claim 16, further including an effective amount of at least one active ingredient selected from antibiotics, antiseptics, other growth promotants, anaesthetics, and mixtures thereof.

18. A pharmaceutical or veterinary composition for the treatment of gastrointestinal injuries, diseases or ulcers, which composition includes

an effective amount of a milk product extract composition including a plurality of cell growth promoting factors, extracted from milk product in concentrated form; said factors having basic to approximately neutral isoelectric points; and

a pharmaceutically or veterinarily acceptable diluent, carrier or excipient therefor.

19. A composition according to claim 18, further including an effective amount of at least one active ingredient selected from antibiotics, antiseptics, other growth promotants, anaesthetics, and mixtures thereof.

20. A method of treating surface wounds in animals, including humans, which method includes administering to the patient to be treated an effective amount of a pharmaceutical or veterinary composition, which composition includes:

an effective amount of a milk product extract composition including a plurality of cell growth promoting factors, extracted from milk product in concentrated form; said factors having basic to approximately neutral isoelectric points; and

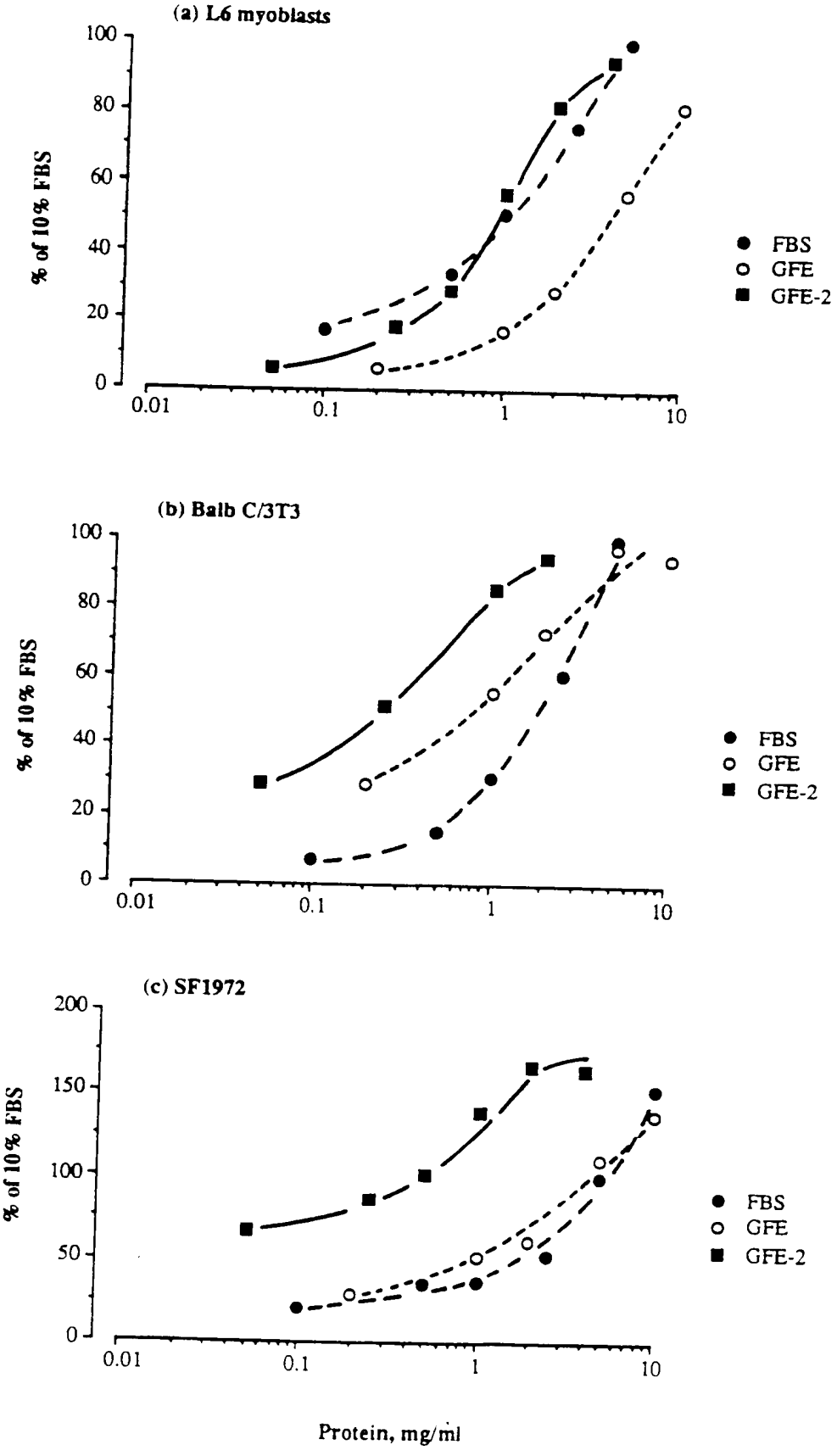
a pharmaceutically or veterinarily-acceptable diluent, carrier or excipient therefor.

21. A method for the treatment of gastrointestinal injuries, diseases or ulcers, which method includes administering to the patient to be treated an effective amount of a pharmaceutical or veterinary composition, which composition includes

an effective amount of a milk product extract composition including a plurality of cell growth promoting factors, extracted from milk product in concentrated form; said factors having basic to approximately neutral isoelectric points; and

a pharmaceutically or veterinarily acceptable diluent, carrier or excipient therefor.

22. A milk product extract composition substantially as hereinbefore described with reference to any one of the examples.


(a) L6 myoblasts
% of 10% FBS
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40
20
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0.1
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FBS
GFE
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(b) Balb C/3T3
% of 10% FBS
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80
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40
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GFE
GFE-2
(c) SF1972
% of 10% FBS
200
150
100
50
0
0.01
0.1
1
10
FBS
GFE
GFE-2
Protein, mg/ml

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent classification (IPC) or to both National Classification and IPC

Int. Cl.[5] C07K 3/02, 3/22, 15/06, C12N 5/06, A23C 21/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C07K 3/22, 15/06 A23C 21/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

AU:IPC as above
Chemical Abstracts : Keywords "Milk", "Cheese", "Whey"

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category*	Citation of Document, [11] with indication, where appropriate of the relevant passages [12]	Relevant to Claim No [13]
X	US,B,4791193 (Shigeo Okonogi et al) 13 December 1988 (13.12.88) see whole document	1,5
X	AU-B-49227/85 (585543) (Snow Brand Milk Products Co Ltd) 26 June 1986 (26.07.86); see whole document especially page 18 lines 7-16	1
X	AU-B-52480/86 (598186) (Synfina-Oleofina S.A.) 14 August 1986 (14.08.86); see page 1 lines 12-16, page 2 line 32 - page 3 line 2	1

(continued)

* Special categories of cited documents : [10]

"A" Document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior t the international filing date but later than the priority date claimed

"T" Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11 October 1991 (11.10.91)	28 October 91
International Searching Authority	Signature of Authorized Officer
AUSTRALIAN PATENT OFFICE	A W BESTOW

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	AU-B-83720/82 (557353) (Societe Nationale Elf Aquitaine) 18 November 1982 (18.11.82); see page 3 lines 9-26 and page 4 line 13-17	1
X	Kirk-Othmer Encyclopedia of Chemical Technology, 2nd Edition, Vol 13 page 562-69	1
X	AU-B-23326/88 (617126) (CIBA-GEIGY AG.) 1 June 1989 (01.06.89), see whole document	1
A	AU-B-67992/74 (476778) (Boehringer Mannheim GMBH) 23 October 1975 (23.10.75); see whole document	5

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4a

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

(1) a milk product extract composition and its method of preparation - claims 1-8
(2) a cell culture composition and a method for culturing cells - claims 9-15
(3) a pharmaceutical or veterinary composition and methods for treating surfaces wounds or gastrointestinal injuries, diseases or ulcers - claims 16-20

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

   1-8

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL APPLICATION NO. PCT/AU 91/00303

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member		
AU 52480/86	BE 901672	CA 1238633	CH 666692
	DE 3603764	DK 572/86	ES 551684
	ES 8704972	FI 860546	FR 2576752
	GB 2171102	IT 1204290	JP 61246198
	LU 86252	NL 8600231	NO 860407
	NZ 214924	SE 8600423	US 4667018
AU 83720/82	AR 228653	BE 893156	CA 1171723
	CH 650905	DE 3218348	DK 2167/82
	FR 2505615	GB 2098998	IT 1151758
	JP 58028233	NL 8201947	SE 8203056
	US 4436658		
AU 49227/85	BE 903886	DE 3544400	FR 2574800
	GB 2168982	JP 61145200	NZ 213985
	US 4668771		
US 4791193	EP 253395	NZ 221082	JP 63152400
AU 67992/74	AR 207222	AT 3167/74	CA 1023353
	CH 580390	FR 2226116	GB 1430490
	IT 1009848	NL 7405082	US 3969337
AU 23326/88	DK 5463/88	EP 313515	PT 88626

END OF ANNEX